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Graft-versus-Host Disease Prophylaxis with Tacrolimus and Mycophenolate Mofetil in HLA-Matched Nonmyeloablative Transplant Recipients Is Associated with Very Low Incidence of GVHD and Nonrelapse Mortality

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Incidence of grade II-IV acute graft-versus-host disease (aGVHD) in nonmyeloablative (NMA) transplant recipients remains high. To date, the ideal prophylaxis regimen, which minimizes aGVHD and chronic GVHD (cGVHD), but does not abrogate graft-versus-tumor (GVT) response, has not been described. Because tacrolimus is more potent than cyclosporine (CSA), and because mycophenolate mofetil (MMF) is an effective immunosuppressant that does not lead to mucositis, we hypothesized that a combination of these 2 oral agents may be an effective GVHD prophylactic strategy. We, therefore, designed an outpatient prospective cohort study with a conditioning regimen consisting of fludarabine (Flu) 30 mg/m^2 daily and cyclophosphamide (Cy) 300 mg/m^2 daily for 5 days followed by infusion of blood stem cells. Tacrolimus 3 mg twice a day was started on day (D) −8, adjusted to achieve levels 10-15 nmol/L, continued until D +50 and then tapered by D +100 or +180 according to estimated risk of relapse. MMF 1000 mg twice a day was started on D +1 and discontinued on D +50. To date, 131 patients (males/females: 75/56) with a median age of 54 years have received a 6/6 matched sibling transplant using this protocol. Indication for NMA transplant included age >55 years (24%), expected increased risk of toxicity (28%), or participation in a multiple myeloma (MM) sequential protocol (48%). Most common diagnoses included MM (N = 62), non-Hodgkin lymphoma (NHL, N = 46), and acute leukemia (N = 10). Following infusion of 6.8×10^6 CD34$^+$ cells/kg (range: 0.30-22.3), neutrophil and lymphocyte engraftment occurred in 95% of patients by D +180. The estimated cumulative incidence of classical grade I-IV aGVHD by D +120 was 11.6% (95% confidence interval [CI]: 7.1-18.5). No grade IV aGVHD was observed. In addition, 15 patients (12%: CI: 7.4-19.2; median D +140) developed an overlap syndrome consisting of clinical and histologic features of both aGVHD and cGVHD simultaneously. The estimated cumulative incidence of extensive cGVHD was 76.1% (95% CI: 67.4-83.9) at 2 years, with clinical features at presentation similar to other reported series. In patients developing extensive cGVHD, the probability of remaining on immunosuppression at 5 years was 34.8% (95% CI: 16.4-57.3). With a median follow-up of 982 days, the estimated probabilities of nonrelapse mortality (NRM) and overall survival (OS) were 15.5% (95% CI: 9.0-26.1) and 62.7% (95% CI: 51.4-72.1). The cumulative incidence of relapse was 30% at 7 years. Following NMA transplant, disease-free survival (DFS) was highest in recipients with follicular NHL (79.8%: 95% CI: 57.6-91.2) and lowest in large cell NHLs (34.3%: 95% CI: 1.6-75.9). From this large group of patients treated with a uniform conditioning and GVHD prophylaxis regimen, we conclude that aGVHD prophylaxis with early use of tacrolimus and MMF is safe, effective, and associated with low NRM. Future strategies will need to focus on decreasing the incidence of extensive cGVHD without increasing the risk of relapse.

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INTRODUCTION

Nonmyeloablative (NMA) allogeneic hematopoietic stem cell transplantation (HSCT) was designed to decrease transplant-related complications and mortality, thus broadening access to transplantation to older and sicker patients [1-5]. Surprisingly, despite a presumably attenuated cytokine storm, the incidence of classical acute graft-versus-host disease (aGVHD) remains high, with reported cumulative incidence of grades II-IV up to 60% [1-5]; serious aGVHD or chronic GVHD (cGVHD) (following NMA transplant) has detrimental consequences in patients including death, disability, infections, or prolonged hospitalization [6,7]. These variations in incidence and severity between series most likely reflect heterogeneity of both transplant populations and of aGVHD prophylaxis regimens.

Despite the large numbers of NMA transplants performed worldwide, there is no consensus yet on the best conditioning and GVHD prophylaxis regimens. An ideal NMA conditioning regimen should be sufficiently immunosuppressive to ensure high engraftment rates and associated with minimal posttransplant toxicity. Posttransplant immunosuppression should be maintained at a level low enough to allow a graft-versus-tumor (GVT) effect, but also sufficient to lead to acceptable rates of aGVHD and cGVHD. We report herein the results of a large, homogenous cohort of 131 patients treated with a novel NMA transplant conditioning and GVHD prophylaxis regimen characterized by outpatient use of fludarabine (Flu) and cyclophosphamide (Cy) with early introduction of tacrolimus to achieve therapeutic levels at time of graft infusion.

MATERIALS AND METHODS

Conditioning Regimen, aGVHD Prophylaxis, and Treatment

The NMA conditioning regimen and immunosuppression withdrawal schedule were designed to permit the GVT effect associated with cGVHD [3] with a view to reduce the incidence of aGVHD. It was also intended to perform the NMA transplant as an outpatient procedure. The conditioning regimen consisted of Cy 300 mg/m^2 intravenously (i.v.) daily and Flu 30 mg/m^2 i.v. daily for 5 days from day (D) -8 to D -4, given from Monday to Friday in our ambulatory facility. Tacrolimus was selected because of its efficacy in preventing aGVHD and its potential role in preventing graft rejection [8-10]. Tacrolimus was started at 3 mg twice a day orally on D -8, and adjusted thrice weekly until D +50 to achieve trough serum levels of 10-15 nmol/L, then tapered by D +100 (high-risk patients) or D +180 (standard-risk patients). Patients with chronic myelogenous leukemia (CML) in chronic phase, acute myelogenous leukemia (AML) in first complete remission (CR1), acute lymphoblastic leukemia (ALL) in CR1, or refractory anemia (with or without ring sideroblasts [RA, RARS]) were considered standard risk; high-risk disease included all others. Donors received granulocyte-colony stimulating factor (G-CSF) 5 μg/kg twice a day for 9 doses and were collected by consecutive daily apheresis until at least $\geq 5 \times 10^6$ CD34$^+$ cells/kg recipient weight had been collected. Allogeneic stem cells were reinfused on day 0 after storage at 4°C overnight; no attempt was made to limit the number of CD34$^+$ cells infused [11]. Mycophenolate mofetil (MMF) was used instead of methotrexate (MTX) to avoid mucositis. It was initiated at 1000 mg twice a day 24 hours after the last infusion of stem cells and discontinued without tapering on D +50. MMF was not adjusted for recipients' weight and levels were not performed. aGVHD and cGVHD were graded according to previously reported criteria after exclusion of infectious or other causes; all efforts were made to obtain a tissue biopsy to confirm diagnosis [12,13]. Distinguishing aGVHD from cGVHD was not restricted to time to symptomatic onset and clinical presentation was considered according to recent consensus criteria [14]. Grade I classical aGVHD was treated with topical steroids only; treatment of grade II-IV classical aGVHD, overlap syndrome, and cGVHD has been described in detail before [15].

Following infusion, all recipients were assessed in the outpatient clinic thrice weekly with appropriate clinical, hematologic, and biochemical evaluations until neutropenia resolved; regular follow-up visits were then undertaken as clinically indicated. Irradiated red blood cells (RBCs) and platelets were transfused if the hemoglobin $\leq$85 g/L or the platelet count was $\leq 15 \times 10^9$/L.

Eligibilty Criteria

This prospective cohort study was conducted at Maisonneuve-Rosemont Hospital, Montreal, Quebec, Canada, a 725-bed tertiary care hospital accredited by the Foundation for Accreditation of Cellular Therapy. All patients were treated and followed at 1 site by the same HSCT team; they were eligible to participate in this protocol if they were 65 years old or less, had a recognized indication for allogeneic transplant according to predetermined institutional criteria for NMA allogeneic transplantation, and a 6/6 HLA compatible sibling donor. Mismatched sibling or unrelated transplant candidates were excluded. Eligible patients were offered treatment on this protocol either because they were too unfit to undergo standard myeloablative (MA) transplant or because they were between the ages of

55 and 65 years. Finally, patients with newly diagnosed multiple myeloma (MM) were invited to participate in a sequential therapy protocol consisting of autologous transplantation followed by NMA transplant. Written informed consent was obtained from all patients after approval by our institutional review board.

Infectious Prophylaxis

Trimethoprim/sulfametoxazole, 1 double-strength tablet twice a day on Saturdays and Sundays, was given for *Pneumocystis jirovecii* prophylaxis starting D −3 and continued until discontinuation of all immunosupppression. Patients who were seropositive for herpes simplex received acyclovir 200 mg three times a day from D −8 until D +21. Cytomegalovirus (CMV) seropositive recipients or recipients transplanted from a CMV-seropositive donor were followed using a preemptive approach of weekly antigenemia or quantitative polymerase chain reaction (PCR) testing from D +14 until D +98, after which routine surveillance was discontinued. Patients with a positive CMV antigen or PCR were promptly treated with i.v. ganciclovir accordingly to a standard algorithm [16]. No other antimicrobial or antifungal prophylaxis was used. Immunoglobulins and G-CSF were not routinely administered after transplant.

Engraftment

Chimerism studies were performed using short variable tandem repeats by PCR assay (GenePrint STR Systems, Promega, Madison, WI) in both lymphocytes and neutrophils every 2 weeks for the first 8 weeks, then monthly for 2 months, and every 3 months for 5 years then yearly thereafter. We defined engraftment (complete donor chimerism [CDC]) when lymphocytes and neutrophils were both ≥95% donor origin. For patients not achieving CDC after D +120 despite withdrawal of imunosuppression and those with persistent or progressive disease, the initial intention was to administer 3 monthly donor lymphocyte infusions (DLIs) of 1×10^7 $CD3^+$ cells/kg each. Because of high incidences of engraftment and cGVHD, DLIs were subsequently given only for persistent or progressive disease.

Statistical Analysis

The primary objective of this prospective cohort study was to determine the incidence and severity of classical aGVHD and cGVHD in patients who received this conditioning regimen and GVHD prophylaxis. Secondary objectives included measurement of engraftment rates, pattern of engraftment in both lymphocytes and neutrophils, duration of cytopenias, transfusion requirements, feasibility to conduct the procedure as an outpatient, and description of outcome according to disease (lymphoma versus MM versus leukemia). Data were analyzed with Stata 8.0 (StataCorp, College Station TX). Proportions were compared with the χ^2 test or, when numbers were small, with Fisher's exact test. Unconditional logistic regression was used for multivariate analysis. Results are expressed as crude (CHR) and adjusted hazard ratios (AHR) with their 95% confidence intervals (CI). Models were built sequentially, starting with the variable most strongly associated with the outcome and continuing until no other variable reached significance or altered the hazard ratios of variables already in the model. Descriptive statistics, Kaplan-Meier (KM) incidence estimates for classical aGVHD and cGVHD, CDC, overall survival (OS), and disease-free survival (DFS) were calculated with the statistical package Prism 4.0 (GraphPad Software, San Diego, CA). OS was defined as time from transplant to death from any cause. DFS was defined as time from transplant to the first event of either relapse or death.

RESULTS

Demographics

Between July 2000 and December 2007, 131 patients were enrolled and followed prospectively after HSCT. Median follow-up is 982 days (range: 80-2576). Patient characteristics are presented in Table 1. Median age was 54 years (range: 20-66 years), and most patients (57%) were male. The most common disease-related indications for transplant were MM (N = 62, 47%) and non-Hodgkin lymphoma (NHL, N = 46, 35%), with most patients having indolent histology (N = 28, 61%). Among MM patients, 54 received planned sequential treatment, whereas 8 were allotransplanted after relapsing from autologous transplant. Other indications for NMA transplant were age ≥55 years (24%) and expected high toxicity with conventional regimen as judged by attending transplant physician (28%). Most patients (60%) had received less than 2 chemotherapy regimens and 87% were in CR or partial remission (PR) prior to NMA. Overall, 101 (77%) had previously received autologous HSCT (average of 10 months; range: 2-135). Less than half (42%) of recipients were at risk for CMV disease; 26% were males who received a graft from a female donor. G-CSF was used in only 22 patients (16%) for a median of 3 days (range: 1-7) for neutropenic fever (N = 3), drug induced neutropenia (N = 2), pneumonia (N = 1), or prevention of neutropenic fever in frail patients (N = 16).

Grafts and Engraftment

The median numbers of infused mononuclear and $CD34^+$ cells were 9.6×10^8/kg (range: 1.2-23.6) and 6.8×10^6/kg (range: 0.30-22.3), respectively. One

Table 1. Shown Are Demographics of 131 Patients Who Received Outpatient Nonmyeloablative (NMA) Transplant

	N = 131 (%)
Age (years)	
Median: 54 (range: 20-66)	
Sex	
Male	75 (57)
Diagnosis	
Multiple myeloma	62 (47)
Sequential protocol	54 (41)
Relapsed	8 (6)
Non-Hodgkin lymphoma	46 (35)
Low grade	28 (21)
Large cell	10 (8)
Mantle	7 (5.3)
Sezary	1 (0.7)
Acute myelogenous leukemia	8 (6)
Acute lymphoblastic leukemia	2 (1.5)
Myelodysplastic syndrome	4 (3)
Chronic myelogenous leukemia	2 (1.5)
Chronic lymphocytic leukemia	4 (3)
Hodgkin disease	3 (2.3)
Indication of NMA transplant	
Age	31 (24)
Expected toxicity	37 (28)
Sequential protocol Prior regimens	63 (48)
0-2	78 (60)
3-5	41 (31)
6-9	12 (9)
Clinical status at NMA transplant	
Complete remission	49 (37)
Partial remission	66 (50)
Stable	9 (7)
Progressive disease	7 (5)
Donor/recipient CMV status	
−/−	48 (37)
−/+	28 (21)
+/−	28 (21)
+/+	27 (21)
Donor/recipient sex pairs	
Male → Male	41 (31)
Male → Female	30 (23)
Female → Male	34 (26)
Female → Female	26 (20)

CMV indicates cytomegalovirus.
Patients with a diagnosis of follicular, small lymphocytic, and lymphoplasmacytic lymphoma are included in the low-grade category.

donor with mobilization failure (collection of 0.30×10^6/kg $CD34^+$ cells) required emergency bone marrow harvest and 1 heavily transfused patient (>30 transfusions) with myelodysplastic syndrome (MDS) received equine antithymocyte globulin (ATG) 10 mg/kg for 3 days in addition to Flu and Cy. In total, 11 patients were unavailable for engraftment analysis, either because of early progression/death (before D +90) with transfer to palliative care or lost to follow-up (N = 1); median time of death for these 11 patients was D +138 (range: 80-190). In the remaining 120 patients evaluable for engraftment, almost 95% (N = 114) achieved CDC by D +180 (Figure 1). We observed no statistically significant difference in time to engraftment between lymphocytes and neutrophils. There were 16 patients who received DLIs, including 5 for mixed chimerism (days +107, +108, +111, +156, and +265) and 11 for progressive disease. To

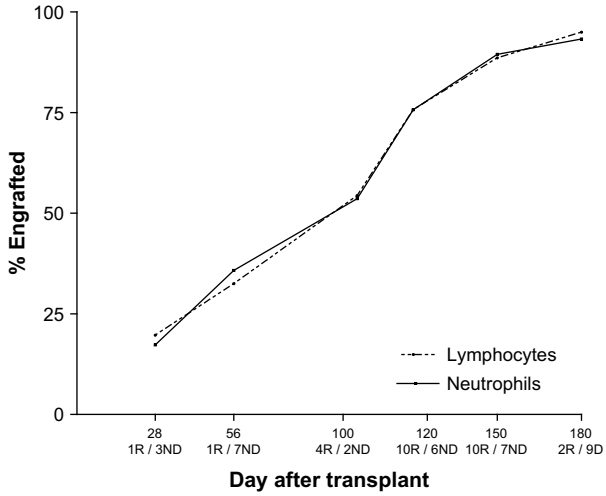

Figure 1. Shown are patients who achieved ≥95% donor cells in granulocytes and lymphocytes following NMA transplant. Patients with either relapse (R), missing specimens (ND), or who died (D) were excluded from denominators.

date, we report no case of late graft failure. Neutropenia defined as $\leq 0.2 \times 10^9$/L or $\leq 0.5 \times 10^9$/L was observed in 57% (median duration 3 days; range: 0-18 days) and 80% (median duration 10 days; range: 2-27 days) of the 131 patients, respectively. Platelet counts $\leq 20 \times 10^9$/L was seen in only 6% (median duration 1 day; range: 0-10 days). Packed RBC transfusions were administered to 40 (31%) patients (median of 3 units; range: 1-26), and platelet transfusions to 17 (13%) patients (median 10 units; range: 5-30).

GVHD

At a median of 69 days (range: 31-129 days), 15 patients developed grade I-IV classical aGVHD (cumulative incidence 11.6%; 95% CI: 7.1-18.5), including 5 grade I, 7 grade II, 3 grade III, but no grade IV (Table 2). Skin and gastrointestinal (GI) involvement was present in 13 (87%) and 4 patients (27%) respectively; no patient experienced hepatic aGVHD. In addition, 15 patients (Table 3) developed an overlap syndrome (cumulative incidence 12%; 95% CI: 7.4-19.2) with clinical and histologic features of both acute (diarrhea ≥500 mL/24 hours) and cGVHD at a median of 140 days (range: 92-177). Interestingly, all patients who underwent colonic biopsy had histologic changes consistent with aGVHD. Liver involvement manifested by either hyperbilirubinemia (N = 3) or increased liver enzymes (N = 6). Altogether, the cumulative incidence of classical aGVHD (grade II-IV) and overlap GVHD was 19.7% (95% CI: 13.7-27.7; Figure 2). Infusion of $\geq 10 \times 10^6$ $CD34^+$/kg and achieving full CDC by D +28 were significant risk factors for development of grade II-IV acute/overlap syndrome in univariate analysis (Table 4). Following multivariate analysis, only achievement of full engraftment by D

Table 2. Shown Are Day of Onset and Organ Stage in 15 Patients Who Presented with Classic Acute Graft-versus-Host Disease

Patient	Day of Onset	Organ Stage			Overall Grade
		Skin	Gastrointestinal	Liver	
1	31	2	0	0	I
2	35	2	0	0	I
3	36	3	0	0	II
4	49	0	3	0	III
5	50	1	0	0	I
6	53	2	0	0	I
7	59	3	0	0	II
8	69	3	0	0	II
9	70	2	0	0	I
10	76	2	3	0	III
11	80	3	0	0	II
12	90	3	0	0	II
13	96	3	0	0	II
14	113	1	1	0	II
15	128	0	3	0	III

+28 remained significant: patients who achieved full engraftment by D +28 were 3 times more at risk of suffering from classical aGVHD or overlap syndrome ($P = .018$).

In contrast, cumulative incidence of extensive cGVHD was 76.1% at 2 years (N = 87, 95% CI: 67.4-83.9%). Day of presentation ranged from 83 to 1146 (median 153 days). Most frequently involved organs included mouth (100%), skin (89%), and liver (65%). The following variables were examined for potential risk factors of extensive cGVHD: age at transplant, sex, indication for transplant, infused $CD34^+$ cells, donor/recipient CMV status, chimerism status at D +28. Intriguingly, the only significant risk factor for development of extensive cGVHD was infusion of $<10.0 \times 10^6$ $CD34^+$/kg cells (CHR 1.9, 95% CI: 1.1-3.3, $P = .03$). The probability of taking any immunosuppressive medication after NMA transplant decreased steadily over time and is estimated at 34.8% (95% CI: 16.4-57.3) 5 years after HSCT (Table 5).

Table 3. Clinical Presentation in 15 Patients with an Overlap Syndrome Consisting of Diarrhea ≥ 500 mL/Day and Clinical/Histological Features of Chronic GVHD Involving Eyes (Sicca Syndrome), Mouth (Lichen Planus), Skin (Erythematous Maculopapular Rash), Liver (Cholestasis, Increased Liver Enzymes), Joints (Synovitis), and Lungs (Bronchiolitis Obliterans)

Patient	Day of Onset	Eyes	Mouth	Skin	Liver	Joints	Lungs
1	92		X	X	X		
2	113		X				
3	121	X	X	X	X		
4	127		X				
5	130		X	X	X		
6	138	X	X	X	X		
7	139		X	X	X		
8	140		X	X	X		
9	140		X	X			
10	140		X	X			
11	148	X	X	X	X		
12	150		X				
13	153		X		X	X	
14	163		X	X	X		
15	177		X	X			X

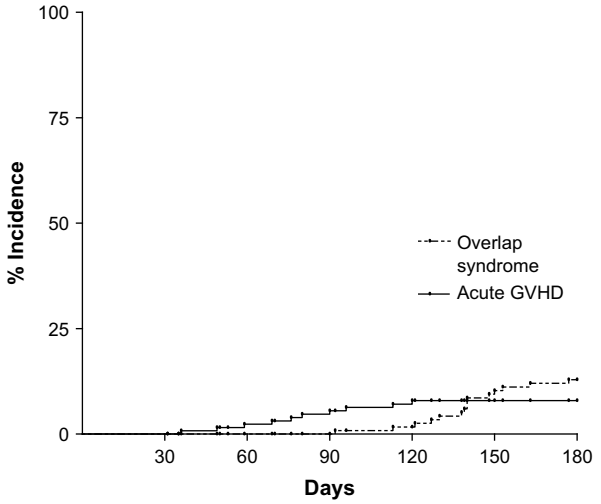

Figure 2. Compares incidences of classic grade II-IV aGVHD (7.7 %) and overlap syndrome (12 %).

Transplant-Related Complications and Outcome

Over the study period, 37 patients died: 24 from relapse and 13 from other causes. With a median follow-up of 982 days (range: 80-2576 days), we observed a nonrelapse mortality (NRM) of 15.5% (95% CI: 9.0-26.1) at 7 years (Figure 3). Other causes of death included complications directly related to cGVHD (n = 3), fulminant hepatitis, disseminated aspergillosis, adult respiratory distress syndrome (ARDS) of unclear etiology, encephalopathy of unknown cause, ruptured aortic aneurysm, anterior myocardial infarction, legionellosis, and massive intraabdominal hemorrhage secondary to anticoagulation. No patient died of aGVHD.

OS for the entire cohort at 1, 3, and 7 years was 88.6% (95% CI: 81.7-92.9), 73.4% (95% CI: 63.9-80.8), and 62.7% (95% CI: 51.5-72.1), respectively (Figure 4), with a median follow-up for survivors of 40.5 months (range: 11-86). Patients with follicular NHL had the best OS at 3 years (78.7%; 95% CI: 55.2-90.8), similar to patients with large cell NHL (67.5%; 95% CI: 16.2-91.9) and MM (73.1%; 95% CI: 58.4-83.3) (Figure 5). In contrast, DFS at 3 years was clearly best for follicular NHL at 78.7% (95% CI: 55.2-90.8), significantly higher than for patients with MM (47.6%, 95% CI: 33.3-60.5, CHR 2.7, $P = .04$) and leukemia/MDS (42.9%, 95% CI: 17.8-66.0, CHR 4.7, $P = .007$); DFS at 3 years was also best for patients with follicular NHL when compared to large cell NHL (34.3%, 95% CI: 1.6-75.9) or other types of NHL (75%, 95% CI: 12.8-96.1, Figure 6).

Overall, incidence of CMV viremia was 31.2%; 29 of 55 (53%) CMV seropositive recipients developed

Table 4. Recipients' Risk Factors for Classic Grade II-IV Acute GVHD and Overlap Syndrome (6 Months Follow-up)

	Patients with GVHD/Total	Incidence (%) of GVHD	Crude Hazard Ratio (95% CI)	Adjusted Hazard Ratio (95% CI)*
Cohort overall	25/131	19.7		
Age at time of transplant (years)				
20-49	9/39	24.5	1.00	1.00
50-59	11/57	19.6	0.85 (0.35-2.04)	0.80 (0.33-1.94)
≥60	5/35	14.8	0.64 (0.22-1.92)	0.68 (0.23-2.03)
Sex				
Female	11/56	20.1	1.00	1.00
Male	14/75	19.4	0.95 (0.43 - 2.08)	1.15 (0.51-2.58)
Indication for transplant				
MM	15/62	24.7	1.00	1.00
NHL, low grade	6/28	21.4	0.87 (0.34 – 2.25)	0.77 (0.30-1.98)
NHL, large cell	1/10	10.0	0.37 (0.05-2.79)	0.21 (0.03-1.64)
NHL, other	1/8	12.5	0.50 (0.07-3.78)	0.44 (0.06-3.39)
Leukemia/MDS	1/14	7.1	0.33 (0.04-2.52)	0.14 (0.02-1.15)
Other	1/9	11.1	0.43 (0.06-3.24)	0.43 (0.06-3.24)
Infused CD34+ cells (×106/kg)				
<10.0	16/103	15.1	1.00	1.00
≥10.0	9/28	33.4	2.34 (1.03-5.30)†	2.04 (0.89-4.69)†
Donor/recipient CMV status				
Negative/negative	9/48	19.0	1.00	1.00
Other	16/83	20.0	1.06 (0.47-2.39)	1.15 (0.51-2.61)
Donor/recipient sex combinations				
Female/male	4/34	11.8	0.49 (0.17-1.41)	0.55 (0.19-1.60)
Other	21/97	22.4	1.00	1.00
Chimerism status D +28				
Partial	15/99	15.6	1.00	1.00
Full engraftment	10/28	37.1	2.99 (1.34- 6.66)‡	2.99 (1.34-6.66) ‡

MM indicates multiple myeloma; NHL, non-Hodgkin's lymphoma; MDS, myelodysplastic syndrome; CMV, cytomegalovirus; GVHD, graft-versus-host disease; CI, confidence interval; D, died.
*Adjusted for chimerism status at D +28.
†$P = .09$.
‡$P = .007$.

viremia, whereas primary infection occurred in 9 seronegative patients who received grafts from seropositive donors. Only 3 patients (2 with pneumonia, D +682 and +1053; 1 with CMV colitis, D +930) developed CMV disease with favorable outcome. Herpes zoster infection was commonly observed with an incidence of 35%. Invasive aspergillosis was diagnosed in 13 patients and was directly the cause of death in 2.

The NMA allogeneic transplant regimen described herein was initially designed to be performed on an outpatient basis. Within the first 100 days, 29 patients (22%) were admitted to the hospital, at a median of D +32 (range: 0-95). Reasons for admission included neutropenic fever (n = 7), active infection (n = 12), disease progression (n = 1), aGVHD (n = 2), hemorragic cystitis (n = 2), interstitial pneumonitis (n = 1), neutropenic colitis (n = 1), and infectious enteritis (n = 3). Between D +101 and D +180, 21 additional patients (17% of 129 evaluable patients) were hospitalized (median time D +149; range 123-178) for the following reasons: complications related to GVHD (N = 10), active infection (n = 6), disease progression (n = 2), fulminant hepatitis (n = 1), thrombocytopenic purpura (n = 1), and gastric perforation with septic shock (n = 1). In total, 38 (29%) patients were never hospitalized during the observation period.

DISCUSSION

An effective GVT effect following NMA allogeneic HSCT is well described [1,2,17-22]. NMA regimens are particularly appealing for older or frail patients who are at higher risk of transplant-related complications with myeloablative transplantation; additionally, the ability to perform transplant on an

Table 5. Shown Is the Prevalence of Patients Taking Any Immunosuppression According to Time Posttransplant

Folow-up Completed		1 Year	2 Years	3 Years	4 Years	5 Years	6 Years
Patients followed		112	84	61	38	23	12
Patients taking immunosuppression	Yes	91	61	41	26	8	3
	No	21	23	20	12	15	9
% Taking immunosuppression		81.3	72.6	67.2	68.4	34.8	25.0
Confidence interval		72.8-88.0	61.8-81.8	54.0-78.7	51.3-82.5	16.4-57.3	5.5-57.2

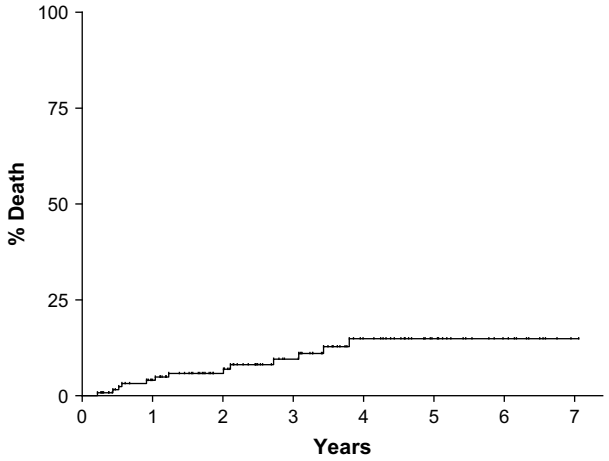

Figure 3. Shows an incidence of non relapse mortality of 15.5% (95% CI: 9.0-26.1) at 7 years in a cohort of 131 patients who received NMA transplant.

outpatient basis remains attractive from both a patient and healthcare utilization perspective [23,24]. aGVHD following NMA transplant is still a challenging problem with a reported incidence between 20% to 60% [2,19,25-27]. GVHD prophylaxis regimens remain currently heterogenous with no consensus regarding the best regimen to use.

Tacrolimus, a calcineurin inhibitor, has interesting properties as an agent for prevention of aGVHD, notably in vitro immunosuppressive effects approximately 100 times higher than cyclosporine A (CsA) [28]; the combination of tacrolimus with MTX has led to a significant reduction in incidence of aGVHD compared to CsA/MTX, with similar toxicity and relapse rates [6,7]. In contrast to MTX, which is well known to cause mucositis [29], MMF has minor mucosal and hematologic toxicities. This advantage has led to replacement of MTX by MMF in a few NMA series [30-34]. Synergy between tacrolimus and MMF in preclinical models [35,36], as well as the negative effects of CsA on MMF trough levels [37,38] motivated us and others [39-41] to study this combination.

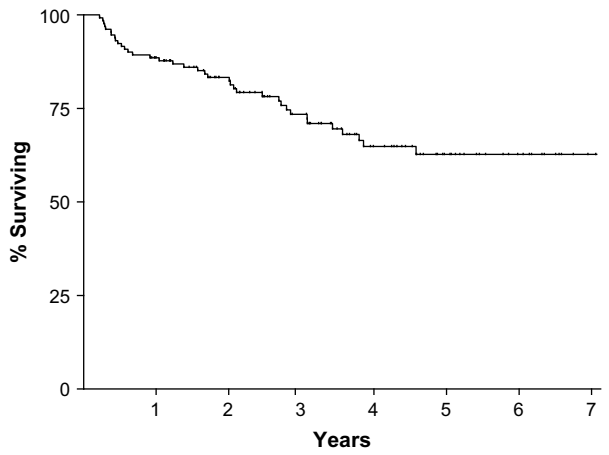

Figure 4. Shows an OS of 62.7% (95% CI : 51.5-72.1) at 7 years for the whole cohort of 131 patients.

This prospective study of 131 patients is the largest published cohort of NMA transplant recipients from a single center who received uniform GVHD prophylaxis using a combination of tacrolimus and MMF. The incidence of classical grade I-IV aGVHD (11.6%) is markedly lower than the incidences of 18.5% and 85% reported by other investigators [40,41], as is the observed incidence of grade II-IV (3.8% versus 15.6% and 42%) and III-IV (1.5% versus 3% and 29%) aGVHD [40,41]. Of note, no grade IV classical aGVHD was observed in this study; intriguingly, skin and gut were the only organs affected with no patient presenting hepatic involvement. Our cumulative incidence of classical aGVHD is also lower than in a series that used CSA/MMF and/or MTX (range: 44%-71%) in the setting of NMA transplant [6,19,22,42-47]. Classical aGVHD occurred at a median of 69 days (range: 31-120 days), similar to other series using tacrolimus/MMF [40], but later than a series using CsA/MMF [45]. Following multivariate analysis, achievement of full chimerism by D +28 was the only significant risk factor for development of grade II-IV aGVHD, a finding observed previously in animal and human studies [18,48,49].

The reasons for such a low incidence of aGVHD and peculiar clinical presentation remain uncertain. The early start of tacrolimus (D −8) in our study compared to D −3 [40] or D +2 [41] might have contributed to the observed lower incidence by earlier achievement of therapeutic levels. Second, inclusion of matched unrelated transplants in Jillella et al's study [41] (25% of patients) might have increased the incidence and severity of GVHD. Third, we speculate that the use of Cy and Flu as NMA conditioning might have attenuated the cytokine "storm," which occurs in the peritransplant period, thereby also contributing to the lower incidence of classical aGVHD.

In addition to a low incidence of classical aGVHD, we observed, similar to the Seattle group, a syndrome of late-onset aGVHD in a minority (12%) of our patients [44]. We elected not to use the term "late-onset aGVHD," because unlike patients described by Mielcarek et al. [44], our 15 patients also had features of cGVHD, a reason we elected to classify them as having an "overlap syndrome." Patients with an overlap syndrome underwent colonic biopsies, which revealed histologic changes consistent with aGVHD [50]. Features of cGVHD across a wide time range (33-832 days after transplant) with histologic findings consistent with aGVHD in biopsied specimens have also been documented in other studies [51,52]. Current consensus is that clinical manifestations, and not time to symptomatic onset after transplantation, determine whether the clinical syndrome of GVHD is considered acute or chronic [14].

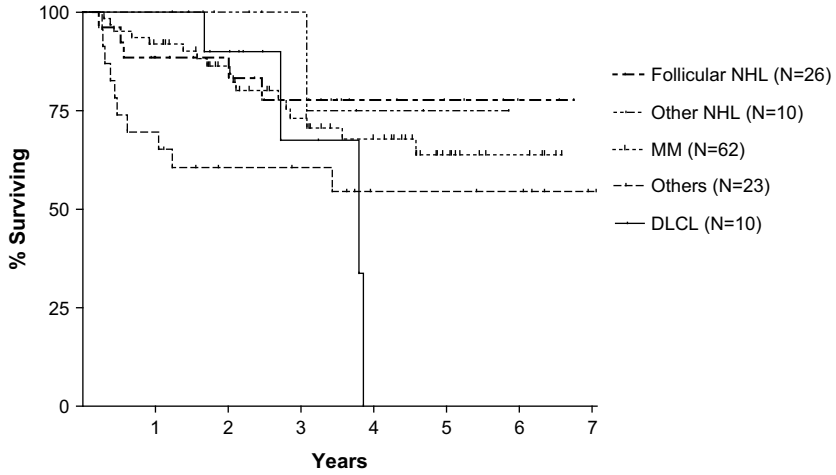

Figure 5. Shown is the estimated OS in our 131 patients according to initial diagnosis. NHL, non Hodgkin lymphoma; MM, multiple myeloma; DLCL, diffuse large-cell lymphoma.

In contrast with our reported very low incidence of classical aGVHD, we observed an incidence of extensive cGVHD of 76% at 2 years, whereas limited cGVHD was present in only a minority (4.6%). Compared to other series using tacrolimus and MMF, our incidence of extensive cGVHD is lower than the 87% reported by Jillella et al. [41], but higher than the 42% reported incidence by Nieto et al. [40]. Similar to our cohort, limited cGVHD was uncommon in both studies (7.7% and 4.3%, respectively). In a mixed cohort using tacrolimus/MMF or CSA/MMF, Rezvani et al. [22] reported a 47% incidence of extensive cGVHD, whereas other studies using CSA/MMF ± MTX reported ranges between 41% and 73% [6,22,42,46,47] with higher incidence if MTX was added [45]. Despite such a relatively high incidence of cGVHD in our cohort, organ involvement at initial presentation was similar to other studies [53,54]. With a median follow-up of 33 months, probability to take immunosuppression in our cohort is also comparable to other reported studies [55].

Many studies have reported at least a trend toward higher incidences of cGVHD following NMA when compared to MA transplant [20,46,56]. One study reported similar [44], whereas another, a lower incidence (14% versus 40%) [57]. An incidence of cGVHD of 76% in our cohort, despite a very low incidence of classical aGVHD, a well-known risk factor of cGVHD, is poorly understood, although some authors have suggested that increased patients' age [46] or using G-CSF mobilized peripheral blood stem cells (PBSCs) might play a role [55]. Our observation of a lower incidence of cGVHD in recipients who have received more $CD34^+$ cells remains unexplained. The change in pattern of GVHD following NMA HSCT might be because of factors such as decreased inflammatory cytokine release [44,58,59], better tolerance resulting from mixed donor-host chimerism [57,60], as well as

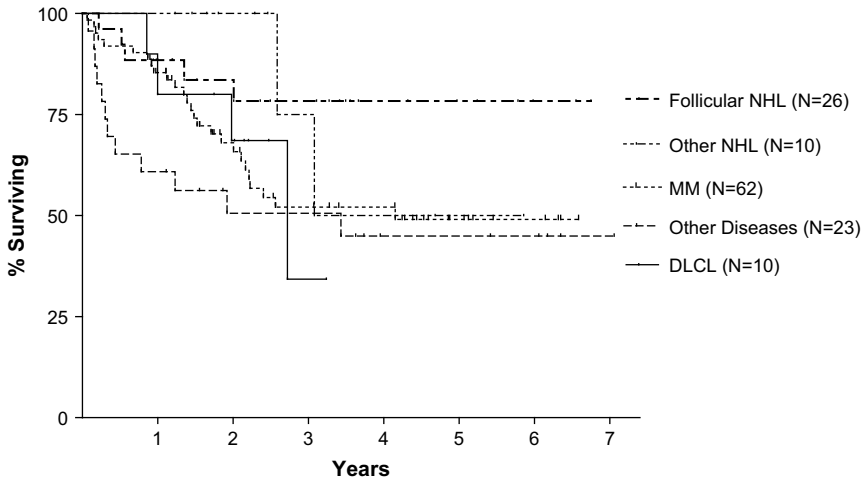

Figure 6. Shown is the estimated DFS in 131 patients according to initial diagnosis. NHL, non Hodgkin lymphoma; MM, multiple myeloma; DLCL, diffuse large-cell lymphoma.

higher numbers of host antigen presenting cells that play a role in initiation of GVHD [58,61].

Our cumulative incidences of NRM and OS (10.2% and 73.4% at 3 years, 15.5% and 62.7% at 7 years) are comparable to that reported by Jillela et al. [41] (7% and 71% at 2.8 years), and Nieto et al. [40] (15.6% at 2 years and 62.5% at 19 months), respectively, with a longer follow-up in favor of our cohort. In contrast, several other studies have reported less favorable outcomes, including Falda et al. (10% and 53% at 1 year), Rezvani et al. (42% and 43% at 3 years), Alyea et al. (32% at 3 years and 51% at 2 years) and Koh et al. (27% at 1 year and 46% at 22 months) [22,26,42,45,62]. Relapse rate at 7 years is 30%, lower than most reported series, ranging from 46% to 53% [26,42,44]. DFS rates for indolent and aggressive NHL are better than what has been reported in other studies [22], but comparable [63] or better than other series of MM [64,65]. The relatively high incidence of cGVHD in our cohort, potentially associated with better GVT effect, might have contributed to lower the mortality rate and improve OS in our patients [66]. Finally, inclusion of less than one-third of patients with aggressive disease subtypes might have contributed to the favorable outcome observed in our cohort.

In conclusion, NMA transplant using a conditioning regimen of Flu/Cy with early introduction of tacrolimus (D −8) followed by addition of MMF for GVHD prophylaxis until D +50 is associated with high engraftment rates (95%), a very low incidence of classical aGVHD, and an overlap syndrome altogether with excellent NRM, OS, and DFS. Our observation of an overlap syndrome with clinical features of both aGVHD and cGVHD strengthens the notion that the traditional D +100 separation of aGVHD and cGVHD should be abandoned and other characteristics such as GVHD-related symptoms and severity be considered in the setting of NMA transplant [6,44,46]. Future strategies to decrease the incidence of cGVHD while preserving antitumor activity are warranted.

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REFERENCES

1. Khouri IF, Keating M, Körbling M, et al. Transplant-lite: induction of graft-versus-malignancy using fludarabine-based nonablative chemotherapy and allogeneic blood progenitor-cell transplantation as treatment for lymphoid malignancies. *J Clin Oncol*. 1998;16:2817-2824.
2. Slavin S, Nagler A, Naparstek E, et al. Nonmyeloablative stem cell transplantation and cell therapy as an alternative to conventional bone marrow transplantation with lethal cytoreduction for the treatment of malignant and nonmalignant hematologic diseases. *Blood*. 1998;91:756-763.
3. Childs R, Chernoff A, Contentin N, et al. Regression of metastatic renal-cell carcinoma after nonmyeloablative allogeneic peripheral-blood stem-cell transplantation. *N Engl J Med*. 2000;343:750-758.
4. Kroger N, Schetelig J, Zabelina T, et al. A fludarabine-based dose-reduced conditioning regimen followed by allogeneic stem cell transplantation from related or unrelated donors in patients with myelodysplastic syndrome. *Bone Marrow Transplant*. 2001;28:643-647.
5. Michallet M, Bilger K, Garban F, et al. Allogeneic hematopoietic stem cell transplantation after nonmyeloablative preparative regimens: impact of pretransplantation and posttransplantation factors on outcome. *J Clin Oncol*. 2001;19:3340-3349.
6. Flowers ME, Traina F, Strorer B, et al. Serious graft-versus-host disease after hematopoietic cell transplantation following nonmyeloablative conditioning. *Bone Marrow Transplant*. 2005;35:277-282.
7. Sala-Torra O, Martin PJ, Storer B, et al. Serious acute or chronic graft-versus-host disease after hematopoietic cell transplantation: a comparison of myeloablative and nonmyeloablative conditioning regimens. *Bone Marrow Transplant*. 2008;41:887-893.
8. Ratanatharathorn V, Nash RA, Przepiorka D, et al. Phase III study comparing methotrexate and tacrolimus (prograf, FK506) with methotrexate and cyclosporine for graft-versus-host disease prophylaxis after HLA-identical sibling bone marrow transplantation. *Blood*. 1998;92:2303-2314.
9. Nash RA, Antin JH, Karanes C, et al. Phase 3 study comparing methotrexate and tacrolimus with methotrexate and cyclosporine for prophylaxis of acute graft-versus-host disease after marrow transplantation from unrelated donors. *Blood*. 2000;96:2062-2068.
10. Hiraoka A, Ohashi Y, Okamoto S, et al. Phase III study comparing tacrolimus (FK506) with cyclosporine for graft-versus-host disease prophylaxis after allogeneic bone marrow transplantation. *Bone Marrow Transplant*. 2001;28:181-185.
11. Dhédin N, Chamakhi I, Perreault C, et al. Evidence that donor intrinsic response to G-CSF is the best predictor of acute graft-vs-host disease following allogeneic peripheral blood stem cell transplantation. *Exp Hematol*. 2006;34:107-114.
12. Shulman HM, Sullivan KM, Weiden PL, et al. Chronic graft-versus-host syndrome in man. A long-term clinicopathologic study of 20 Seattle patients. *Am J Med*. 1980;69:204-217.
13. Przepiorka D, Weisdorf D, Martin P, et al. 1994 Consensus conference on acute GVHD grading. *Bone Marrow Transplant*. 1995;15:825-828.
14. Filipovich AH, Weisdorf D, Pavletic S, et al. National Institutes of Health consensus development project on criteria for clinical trials in chronic graft-versus-host disease: I. Diagnosis and staging working group report. *Biol Blood Marrow Transplant*. 2005;12:945-956.
15. Labbé AC, Su SH, Laverdière M, et al. High incidence of invasive aspergillosis associated with intestinal graft-versus-host disease following nonmyeloablative transplantation. *Biol Blood Marrow Transplant*. 2007;13:1192-1200.
16. Boivin G, Bélanger R, Delage R, et al. Quantitative analysis of cytomegalovirus (CMV) viremia using the pp65 antigenemia assay and the COBAS AMPLICOR CMV MONITOR PCR test after blood and marrow allogeneic transplantation. *J Clin Microbiol*. 2000;38:4356-4360.
17. Giralt S, Estey E, Albitar M, et al. Engraftment of allogeneic hematopoietic progenitor cells with purine analog-containing chemotherapy: harnessing graft-versus-leukemia without myeloablative therapy. *Blood*. 1997;89:4531-4536.
18. Childs R, Clave E, Contentin N, et al. Engraftment kinetics after nonmyeloablative allogeneic peripheral blood stem cell transplantation: full donor T-cell chimerism precedes alloimmune responses. *Blood*. 1999;94:3234-3241.

19. McSweeney PA, Niederwieser D, Shizuru JA, et al. Hematopoietic cell transplantation in older patients with hematologic malignancies: replacing high-dose cytotoxic therapy with graft-versus-tumor effects. *Blood*. 2001;97:3390-3400.
20. Maris MB, Sandmaier BM, Storer BE, et al. Allogeneic hematopoietic cell transplantation after fludarabine and 2 Gy total body irradiation for relapsed and refractory mantle cell lymphoma. *Blood*. 2004;104:3535-3542.
21. Kato K, Khaled Y, Mineishi S. Reduced-intensity stem cell transplantation for hematological malignancies: current status and the future. *Curr Stem Cell Res Ther*. 2007;2:149-162.
22. Rezvani AR, Storer B, Maris M, et al. Nonmyeloablative allogeneic hematopoietic cell transplantation in relapsed, refractory, and transformed indolent non-Hodgkin's lymphoma. *J Clin Oncol*. 2008;26:211-217.
23. Ruiz-Argüelles GJ, Gómez-Almaguer D, Ruiz-Argüelles A, et al. Results of an outpatient-based stem cell allotransplant program using nonmyeloablative conditioning regimens. *Am J Hematol*. 2001;66:241-244.
24. Subirà M, Sureda A, Ancín I, et al. Allogeneic stem cell transplantation with reduced-intensity conditioning is potentially feasible as an outpatient procedure. *Bone Marrow Transplant*. 2003;32:869-872.
25. Khouri IF, Saliba RM, Giralt SA, et al. Nonablative allogeneic hematopoietic transplantation as adoptive immunotherapy for indolent lymphoma: low incidence of toxicity, acute graft-versus-host disease, and treatment-related mortality. *Blood*. 2001; 98:3595-3599.
26. Alyea EP, Kim HT, Ho V, et al. Comparative outcome of nonmyeloablative and myeloablative allogeneic hematopoietic cell transplantation for patients older than 50 years of age. *Blood*. 2005;105:1810-1814.
27. Satwani P, Harrison L, Morris E, et al. Reduced-intensity allogenec stem cell transplantation in adults and children with malignant and nonmalignant diseases; end of the beginning and future challenges. *Biol Blood Marrow Transplant*. 2005;11:403-422.
28. Kino T, Hatanaka H, Miyata S, et al. FK-506, a novel immunosuppressant isolated from a Streptomyces. II. Immunosuppressive effect of FK-506 in vitro. *J Antibiot*. 1987;40:1256-1265.
29. Cutler C, Li S, Kim HT, et al. Mucositis after allogeneic hematopoietic stem cell transplantation: a cohort study of methotrexate- and non-methotrexate-containing graft-versus-host disease prophylaxis regimens. *Biol Blood Marrow Transplant*. 2005;11: 383-388.
30. Basara N, Blau WI, Kiehl MG, et al. Mycophenolate mofetil for the prophylaxis of acute GVHD in HLA-mismatched bone marrow transplant patients. *Clin Transplant*. 2000;14:121-126.
31. Vogelsang GB, Arai S. Mycophenolate mofetil for the prevention and treatment of graft-versus-host disease following stem cell transplantation: preliminary findings. *Bone Marrow Transplant*. 2001;27:1255-1262.
32. Mohty M, de Lavallade H, Faucher C, et al. Mycophenolate mofetil and cyclosporine for graft-versus-host disease prophylaxis following reduced intensity conditioning allogeneic stem cell transplantation. *Bone Marrow Transplant*. 2004;34:527-530.
33. Neumann F, Graef T, Tapprich C, et al. Cyclosporine A and mycophenolate mofetil vs cyclosporine A and methotrexate for graft-versus-host disease prophylaxis after stem cell transplantation from HLA-identical siblings. *Bone Marrow Transplant*. 2005;35:1089-1093.
34. Nash RA, Johnston L, Parker P, et al. A phase I/II study of mycophenolate mofetil in combination with cyclosporine for prophylaxis of acute graft-versus-host disease after myeloablative conditioning and allogeneic hematopoietic cell transplantation. *Biol Blood Marrow Transplant*. 2005;11:495-505.
35. Tanabe M, Todo S, Murase N, et al. Therapeutic synergism between low-dose FK-506 and antimetabolites in rat allogeneic heart transplantation. *Transplant Proc*. 1995;27:364-365.
36. Zucker K, Rosen A, Taroucha A, et al. Unexpected augmentation of mycophenolic acid pharmacokinetics in renal transplant patients receiving tacrolimus and mycophenolate mofetil in combination therapy, and analogous in vitro findings. *Transpl Immunol*. 1997;5:225-232.
37. Gregoor PJ, de Sévaux RG, Hené RJ, et al. Effect of cyclosporine on mycophenolic acid trough levels in kidney transplant recipients. *Transplantation*. 1999;68:1603-1606.
38. van Gelder T, Klupp J, Barten MJ, et al. Comparison of the effects of tacrolimus and cyclosporine on the pharmacokinetics of mycophenolic acid. *Ther Drug Monit*. 2001;23:119-128.
39. McSweeney P, Abhyankar S, Foran J, et al. Favorable early transplant outcomes using tacrolimus and mycophenolate mofetil for GVHD prevention after matched sibling allografting. *Blood*. 2003;102:714a.
40. Nieto Y, Patton N, Hawkins T, et al. Tacrolimus and mycophenolate mofetil after nonmyeloablative matched-sibling donor allogeneic stem-cell transplantations conditioned with fludarabine and low-dose total body irradiation. *Biol Blood Marrow Transplant*. 2006;12:217-225.
41. Jillella AP, Shafer D, Klumpp TR, et al. Mixed chimerism and graft failure following conditioning with the fludarabine and cyclophosphamide nonablative regimen; conversion to full donor chimerism. *Am J Hematol*. 2007;82:419-426.
42. Falda M, Busca A, Baldi I, et al. Nonmyeloablative allogeneic stem cell transplantation in elderly patients with hematological malignancies: results from the GITMO (Gruppo Italiano Trapianto Midollo Osseo) multicenter prospective clinical trial. *Am J Hematol*. 2007;82:863-866.
43. Gupta V, Daly A, Lipton JH, et al. Nonmyeloablative stem cell transplantation for myelodysplastic syndrome or acute myeloid leukemia in patients 60 years or older. *Biol Blood Marrow Transplant*. 2005;11:764-772.
44. Mielcarek M, Martin PJ, Leisenring W, et al. Graft-versus-host disease after nonmyeloablative versus conventional hematopoietic stem cell transplantation. *Blood*. 2003;102:756-762.
45. Koh LP, Chen CS, Tai BC, et al. Impact of postgrafting immunosuppressive regimens on nonrelapse mortality and survival after nonmyeloablative allogeneic hematopoietic stem cell transplant using the fludarabine and low-dose total-body irradiation 200-cGy. *Biol Blood Marrow Transplant*. 2007;13:790-805.
46. Pérez-Simón JA, Díez-Campelo M, Martino R, et al. Influence of the intensity of the conditioning regimen on the characteristics of acute and chronic graft-versus-host disease after allogeneic transplantation. *Br J Haematol*. 2005;130:394-403.
47. Burroughs L, Mielcarek M, Leisenring W, et al. Extending postgrafting cyclosporine decreases the risk of severe graft-versus-host disease after nonmyeloablative hematopoietic cell transplantation. *Transplantation*. 2006;81:818-825.
48. Manilay JO, Pearson DA, Sergio JJ, et al. Intrathymic deletion of alloreactive T cells in mixed bone marrow chimeras prepared with a nonmyeloablative conditioning regimen. *Transplantation*. 1998;66:96-102.
49. Baron F, Baker JE, Storb R, et al. Kinetics of engraftment in patients with hematologic malignancies given allogeneic hematopoietic cell transplantation after nonmyeloablative conditioning. *Blood*. 2004;104:2254-2262.
50. McDonald GB, Shulman HM, Sullivan KM, et al. Intestinal and hepatic complications of human bone marrow transplantation. *Gastroenterology*. 1986;90:460-477.
51. Akpek G, Chinratanalab W, Lee LA, et al. Gastrointestinal involvement in chronic graft-versus-host disease: a clinicopathologic study. *Biol Blood Marrow Transplant*. 2003;9:46-51.
52. Bridge AT, Nelson RP Jr., Schwartz JE, et al. Histological evaluation of acute mucocutaneous graft-versus-host disease in nonmyeloablative hematologic stem cell transplants with an observation predicting an increased risk of progression to chronic graft-versus-host disease. *Am J Dermatopathol*. 2007;29:1-6.
53. Subramaniam DS, Fowler DH, Pavletic SZ. Chronic graft-versus-host disease in the era of reduced-intensity conditioning. *Leukemia*. 2007;21:853-859.
54. Mielcarek M, Storb R. Graft-vs-host disease after non-myeloablative hematopoietic cell transplantation. *Leuk Lymphoma*. 2005;46:1251-1260.

55. Stewart B, Storer B, Storek J, et al. Duration of immunosuppressive treatment for chronic graft-versus-host disease. *Blood*. 2004; 104:3501-3506.
56. Petersen SL, Madsen HO, Ryder LP, et al. Haematopoietic stem cell transplantation with non-myeloablative conditioning in the outpatient setting: results, complications and admission requirements in a single institution. *Br J Haematol*. 2004;125:225-231.
57. Couriel DR, Saliba RM, Giralt S, et al. Acute and chronic graft-versus-host disease after ablative and nonmyeloablative conditioning for allogeneic hematopoietic transplantation. *Biol Blood Marrow Transplant*. 2004;10:178-185.
58. Hill GR, Crawford JM, Cooke KR, et al. Total body irradiation and acute graft-versus-host disease: the role of gastrointestinal damage and inflammatory cytokines. *Blood*. 1997;90:3204-3213.
59. Cooke KR, Hill GR, Crawford JM, et al. Tumor necrosis factor-alpha production to lipopolysaccharide stimulation by donor cells predicts the severity of experimental acute graft-versus-host disease. *J Clin Invest*. 1998;102:1882-1891.
60. Colson YL, Lange J, Fowler K, et al. Mechanism for cotolerance in nonlethally conditioned mixed chimeras: negative selection of the Vbeta T-cell receptor repertoire by both host and donor bone marrow-derived cells. *Blood*. 1996;88:4601-4610.
61. Shlomchik WD, Couzens MS, Tang CB, et al. Prevention of graft versus host disease by inactivation of host antigen-presenting cells. *Science*. 1999;285:412-415.
62. Niederwieser D, Maris M, Shizuru JA, et al. Low-dose total body irradiation (TBI) and fludarabine followed by hematopoietic cell transplantation (HCT) from HLA-matched or mismatched unrelated donors and postgrafting immunosuppression with cyclosporine and mycophenolate mofetil (MMF) can induce durable complete chimerism and sustained remissions in patients with hematological diseases. *Blood*. 2003;101: 1620-1629.
63. Kroger N, Sayer HG, Schwerdtfeger R, et al. Unrelated stem cell transplantation in multiple myeloma after a reduced-intensity conditioning with pretransplantation antithymocyte globulin is highly effective with low transplantation-related mortality. *Blood*. 2002;100:3919-3924.
64. Giralt S, Aleman A, Anagnostopoulos A, et al. Fludarabine/melphalan conditioning for allogeneic transplantation in patients with multiple myeloma. *Bone Marrow Transplant*. 2002;30: 367-373.
65. Mohty M, Boiron JM, Damaj G, et al. Graft-versus-myeloma effect following antithymocyte globulin-based reduced intensity conditioning allogeneic stem cell transplantation. *Bone Marrow Transplant*. 2004;34:77-84.
66. Baron F, Maris MB, Sandmaier BM, et al. Graft-versus-tumor effects after allogeneic hematopoietic cell transplantation with nonmyeloablative conditioning. *J Clin Oncol*. 2005;23: 1993-2003.